Regeneration in the Lower Metazoa

by

Mary Frances Goffin

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Thesis

REGENERATION IN THE LOWER METAZOA

by

Mary Frances Goffin

(B. A., Seton Hill College, 1941)

submitted in partial fulfillment of the

requirements for the degree of

Master of Arts

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TOPICAL OUTLINE.

LIST OF ILLUSTRATIONS.

INTRODUCTION.

The first experiments to attract general attention to the problem of regeneration were the observations and experiments of Abbé Trembley on hydras in 1740, although a few cases of regeneration were spoken of by Aristotle and Pliny. Trembley tried to find out if hydras were plants or animals by cutting them into pieces, because at that time it was known that pieces of a plant made new plants, but if an animal was cut into pieces it would die. However, on cutting hydras in two, he found that two hydras were produced; but from his other observations on the methods of feeding and on movement, he concluded that the hydra was an animal and that the property of the development of a new organism from a part must belong to animals as well as plants. Following these experiments, Reamur in 1742, and Bonnet in 1745, investigated regeneration in starfish and fresh water worms, publishing their results and thus arousing widespread interest in the problem. Many different kinds of animals were experimented with to test their powers of regeneration. Spallanzani used a number of different animals, but a complete account of his work was never published, and only the abstract is given in his Prodromo in 1768.

The experiments of Trembley, Reamur, Bonnet, and Spallanzani furnished the basis of later work. The problem of regeneration has been probed much deeper, but many important

facts in regard to regeneration have their foundation on the work of these four pioneers in the field. The various phyla of invertebrate animals have been investigated with respect to their regenerative powers in the hope of finding the solution for the phenomenon of regeneration. This thesis will deal with the description of regeneration, and the factors relating to regeneration of the lower invertebrate groups, namely, the sponges, the coelenterates, the ctenophores, and the flatworms.

The problem of regeneration involves several aspects. Why does mutilation of an organism give rise to the phenomena of growth which does not occur without mutilation? Why does the new growth frequently result in a restoration of the old form of the mutilated organism? Another aspect deals with the nature of the stimulus and involves types of responses made by the tissues to the stimulus.

Several investigators have presented definitions in an attempt to explain the phenomenon of regeneration. Barfurth (1901) defined regeneration as a process in which there is a replacement of the organized whole from a part of the organism. He suggested that if a part is given by nature there follows a process of physiological regeneration. If the part regenerated is the result of artificial injury the result is pathological regeneration. This definition is not wholly true, because there is nothing pathological in the process, as is shown in hydra, where a piece is transformed directly into a new organism. That the process is not strictly a pathological

one is shown in Planaria, where a new head is formed when various cuts are made. Morgan (1901) found Hertwig's definition of regeneration as the power of replacement of a part of an organism, to be incomplete. Morgan stated regeneration was not only the replacement of a lost part, but also the development of a new whole organism, or even part of an organism, from a piece of an adult, or an embryo, or even an egg.

Regeneration in animals is outlined (Morgan, 1901) as occurring in several different ways. Epimorphosis is the name given to those cases in which a proliferation of material precedes the development of the new part. Morphallaxis is the type in which the part is transformed directly into the new organism or part of an organism, with a proliferation of cells at the cut surfaces. This type can be further subdivided into the homomorphosis type and the heteromorphosis type. The homomorphosis type is that in which the new part is like that removed, or like a part of that removed. This type is further subdivided into a halomorphosis type in which the entire lost part is replaced at once or later, and meromorphosis, in which the new part is smaller than the part removed. Heteromorphosis is defined as those cases in which the new part is different from the part removed. Again this is more minutely described by neomorphosis, a type in which the new part is different from the part removed, but is also an organ that belongs to a different part of the body.

There is a constant interchange of material and of energy between an animal and it's surroundings. The environmental

factors in regeneration are influenced by temperature, light, gravity, contact, and food. Temperature influences the rate at which regeneration will take place. The optimum temperature is described as that temperature at which the greatest number of regenerants are obtained. Many times a low temperature will inhibit the process of regeneration.

In some animals deprived of food external regeneration may take place. In cases of this type, material for the new part is derived from an excess of material in the old part. The protoplasm itself appears to be drawn upon to furnish material for the new part. Regeneration occurs very slowly.

Light also seems necessary for regeneration in many of the animals.

The only case known among animals in which regeneration is influenced by gravity is in the case of the hydroid Antennularia. The action of the gravity has not been determined.

In some of the hydroids, portions of the stem touching the glass dish, or other objects, regenerate stolons wherever contact occurs.

Internal factors within the body of the animal regulate regeneration. These factors depend to a great extent on the nature of the stimulus and the response of the tissues to the stimulus. The response of the tissues to stimuli may be manifested in a number of different ways such as lateral regeneration, anterior-posterior regeneration, oblique regeneration, or transformation.

Chemical factors also have an influence upon regeneration. Organisms that live in the water may be affected by the quantity and kinds of salts contained in the water, by dissolved gases, and by the oxygen consumption. Organisms may also be affected by the components of the atmosphere.

PHYLUM PORIFERA.

The Porifera are the simplest multicellular animals and may be characterized as Metazoa with tissues but without organs. The members of this phylum have a more or less extensive system of canals and pores, but no digestive cavity or enteron. The skeleton of these animals is made up of spicules.

From budding and growth, which occur so extensively in these animals, it would appear that they are capable of great powers of regeneration. However, some species will regenerate after cutting and dissociation, but in the majority of species the restoration of parts is limited.

From studies on the regeneration of sponges, investigators have tried to answer various questions. Do the cells dedifferentiate and transform into other types of tissue elements by losing their cytoplasmic individuality through intercellular connections? Is there only temporary cellular differentiation, the individual cells always resuming their original and unchangeable character? Have the undifferentiated cells varying degrees of regenerative powers which make them capable of restoring typical structures after injury? In an attempt to answer these questions the process of regeneration has been studied extensively in Microciona prolifera, a common red sponge along the Atlantic Coast.

The tissues of the normal sponge are the epidermis and the mesenchyme. The epidermis was found by Wilson (1930) to be made up of epithelial cells or pinacocytes. The epidermal

sheet as a whole appeared granular when sections were stained with haemalum.

Making up the mesenchyme of the normal sponge are a number of different types of cells. Wilson (1930) observed nucleolate cells having a large spheroidal nucleus and a large nucleolus which had spheroidal inclusions of an orange color and solid masses in it. These cells were amoeboid in their movement and occurred in abundance throughout the mesenchyme. They were least abundant in the dermal membrane (the layer which separates the superficial canal spaces from the exterior and is made up of epidermis, mesenchyme, and the epithelioid membrane lining the canals).

Gray cells were also found to be abundant in the mesenchyme. These cells were non-nucleolated and contained abundant gray granules which were circular in outline and filled the cell.

Another cell type, the rhabdiferous cell, was common in the mesenchyme. Cells of this type were found in the dermal membrane, and close to the epitheloid membranes lining the canal spaces. The cells almost always appeared elongated in shape and had elongate, narrow inclusions in their protoplasm. The nuclei of the rhabdiferous cells were non-nucleolated.

Also found in the mesenchyme of normal sponges were the globoferous cells. These were described by Wilson as being either elongated and narrow in shape, or spheroidal. The nuclei of these cells were small and non-nucleolated. The

cells were found to be abundant in the dermal membrane.

Fiber cells, another type of mesenchymal cells, were elongated, narrow, spindle-shaped cells, found abundantly throughout the dermal membrane. The nuclei of these cells were granular and longer than they were wide.

Spongoblasts and scleroblasts also occurred in abundance throughout the mesenchyme of normal sponges.

DISSOCIATION. Upon dissociation the tissues are broken up into a number of different types of cells each contributing to the regeneration of a whole individual. According to Galtsoff (1925b) regeneration after dissociation may consist in the sorting out of already differentiated cells which come to occupy their former positions as in the normal organism.

Wilson's method of dissociation was to press the sponge through fine silk gauze of about 300 micra. In some cases the tissue fell through the air onto slides to be studied in reunition; in other cases, the bag was immersed in water then the tissue was collected, later fixed in Bouin's, sectioned and stained with haemalum, methylene blue or Nile sulphate.

The dissociated tissue yielded a variety of results. The most abundant cells were the nucleolate cells as described by Wilson, and called by Galtsoff (1925a) the archaeocytes or the unspecialized granular cells of the mesenchyme. Wilson found that after expression most of the cells were spheroidal in shape and frequently contained bright red granules which were also found present in the collar cells.

The gray cells as described by Wilson (1930), and which were called by Galtsoff in an earlier paper (1925a) the collenocytes, were also found in abundance in the expressed tissue.

Wilson also found rhabdiferous cells in the expressed tissue. These corresponded to Galtsoff's desmacytes. They were found to occur much less abundantly than the other types of cells. No entire globoferous cells were described or observed in the expressed material by either Wilson or Galtsoff.

A type of cell, the psuedo-collar cell, was found by Wilson (1930) as being very abundant in suspension, but did not occur at all among the dissociated cells. He thought they might possibly be the ciliated cells of the embryo. In the living preparations he observed them uniting with small aggregates. Their identity and nature are still uncertain.

The collar cells (Galtsoff's choanocytes) were found by Wilson (1930) to be abundant in the expressed tissue, although Galtsoff had previously (1925a) claimed to have found none in suspension. These were described by Wilson as having a clear cytoplasm with bright red subspheroidal granules in it. He found the nucleus was not visible. He observed individual isolated cells each still retaining a flagellum which was two to three times the length of the cell. Good preparations often showed a thin transparent film projecting from the body through which the base of the flagellum could be seen. He interpreted this structure to represent the collar.

Galtsoff (1925a) described cells which he called

pinacocytes as being abundant in the dermal membrane. The cells had a clear protoplasm, were free of granules and contained a small nucleus without a nucleolus. Wilson, however, claimed these cells were really the collar cells.

The role of the separated cells of Microciona are clearly shown by a diagram taken from Galtsoff (1925b) and reproduced in Figure 1 on this page.

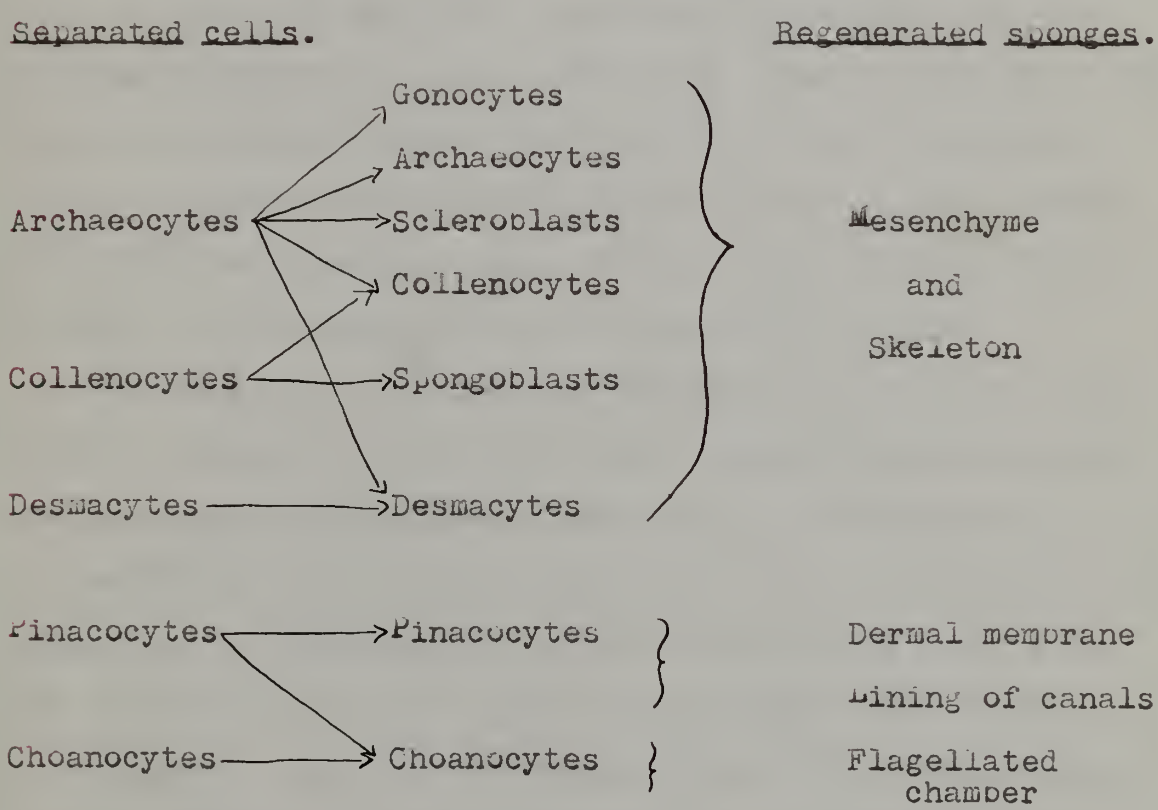

Figure 1.

The Role of Separated Cells in Regeneration.

REUNITION. Tissue strewn on slides and cultured to form reunition masses was described by Wilson (1930) as not undergoing any change until it had become firmly attached to the substratum. This occurred eighteen to twenty-four hours after sowing on the slides. At the end of this time the masses had flattened and spread. Galtsoff found that the flattening and spreading was absolutely necessary for the development of the aggregate and was due to the slow movement of the archaeocytes back and forth on the glass alternately expanding and contracting themselves. Upon coalescence changes took place and those archaeocytes immediately in contact with the glass spread out and became isolated from the common mass. This spreading, he stated, was due to "molecular forces" acting between the protoplasm and the underlying surfaces.

By the end of the third day metamorphosis had been completed. Galtsoff (1925) found that the archaeocytes played the most important role in regeneration, differentiating into scleroblasts, collenocytes (which gave rise to spongioblasts), desmacytes and choanocytes. Those archaeocytes which gave rise to the spicules and formed the skeleton were called scleroblasts. The collenocytes were described as taking part in the formation of the periphery of the aggregate. The pinacocytes differentiated into choanocytes and formed the dermal membrane and the lining of the walls of the canals. Thus the reunition mass had become a thin incrusting sponge with a functional canal system on the third day after dissociation.

The formation of the aggregate, Galtsoff (1923) found, was due to the amoeboid activity of the archaeocytes which extended hyaline psuedopodia. The cells united with other cells by their amoeboid movement, coalesced, and eventually an aggregate formed. The movement of the archaeocytes was irregular and more or less accidental. The ectoplasmic layer of the archaeocytes was fluid and sticky, thus enabling the cells to adhere to objects and to each other. Huxley (1921) had previously stated that the cells do not coalesce as a result of chance, but there may be a definite attraction between cells.

On the third day of development a group of small cells located at the thickest parts of the aggregate appeared. Wilson(1930) believed these to be analogous to the flagellated chambers. On the fourth day of development collars and flagella appeared. On the fourth and fifth days canals appeared as splits, arising independently, in the mesenchyme. Then development after dissociation was completed.

TEMPERATURE. At low temperature it was found by Galtsoff (1925a) that the archaeocytes moved more slowly. Therefore, regeneration occurred more slowly.

OSMOTIC PRESSURE. The dissociated cells were found by Galtsoff (1925a) to be more sensitive to an increase of osmotic pressure than to a decrease. The lowest concentration at which coalescence was found to take place was between twelve and four tenth percent and nine and four tenth percent. When the salinity had dropped to nine and four tenth percent the cells underwent cytological changes and disintegrated.

EFFECTS OF ELECTROLYTES. In as pure solutions of sodium or potassium chloride as could be obtained, the cells did not move, according to Galtsoff (1925a), and coalescence did not take place. At the end of twenty-four hours, the solution was changed and the cells transferred to normal sea water. Some of the cells recovered and formed aggregates, although coalescence was incomplete. When sodium chloride and potassium chloride were mixed the same effect was found. The cells died within two days. However, the sodium chloride and potassium chloride could be counteracted by calcium and magnesium ions in amounts corresponding to their contents in sea water. This brought about a coalescence of the cells and the formation of aggregates.

In pure isotonic sodium chloride or potassium chloride solutions or mixtures of them, the amoeboid movement of the cells was inhibited and the cells failed to form aggregates. The inhibitive action of the monovalent sodium and potassium ions was counteracted by the addition of the bivalent calcium and magnesium ions. The amoeboid movement and coalescence took place in pure isotonic magnesium chloride solutions. Pure isotonic calcium chloride solution instantly killed the cells.

MIXTURE OF TWO DIFFERENT SPECIES. When two different species were mixed in normal sea water to form aggregates, as Galtsoff (1923) tried with Microciona and Cliona, it was found that cells coalesced only with cells of their own species. However, when the alkalinity of the sea water was

raised, the aggregates of different species stuck to one another, but the mass soon disintegrated. The cells that adhered to one another and produced the regenerated form were always cells of the same species.

<u>FRESH WATER SPONGES</u>. Penny (1933) found that fresh water sponges when placed under conditions unfavorable for their normal behavior lost their organization and formed small compact bodies of cells or reduction masses. If left in this condition they would die, but when these reduction bodies were placed in a favorable environment they would reform new sponges in much the same manner as already described for Microciona.

PHYLUM COELENTERATA.

The coelenterates are the simplest multicellular animals and are without most of the organs and tissues which characterize the highest animals. They are radially symmetrical animals composed of two layers of cells, the ectoderm and endoderm, and an intermediate gelatinous layer called mesoglea. There is a single opening functioning as mouth and anus, which is usually surrounded by tentacles.

HYDRA.

The hydras are elongate, cylindrical animals able to attach themselves temporarily by means of a sticky secretion at one end which may be called a foot. This is also used in locomotion. There is a mouth and tentacles. No perisarc is present.

As has been already mentioned, the earliest experiments on regeneration were performed by the Swiss priest Trembley (Morgan, 1901) by cutting Hydra viridis. When a hydra was cut in two, two new hydras were produced. If a hydra was cut into three or four parts, each piece produced a new individual. If these were fed and cut into more pieces, each produced new polyps behaving as ordinary polyps. Further experiments involving cuts in the anterior end with the tentacles were tried, and new animals were formed. If only the head end of a hydra was split in two, each half produced a new head, and a two headed hydra resulted. If this were split again

the result was a four headed hydra. Each behaved as separate individuals although all were united on the same stalk. Trembley went still further in his regeneration experiments and was the first worker to observe that a hydra, turned inside out, might regenerate. By pushing the foot through the mouth, he maintained that the ectoderm which lines the entire gastrovascular cavity came to take over the function of the endoderm, and the endoderm assumed the functions of the cells. Roudabush (1933) repeated this experiment of Trembley in order to determine the changes occurring in the ectoderm and endoderm after the position of the two layers had been reversed. Using Hydra viridis, Hydra vulgaris, and Hydra oligactis as material, he prodded the hydras with a needle until they were stimulated to contract. When fully contracted, the anterior end was placed on the bottom of a culture dish and the basal disk was pushed through the mouth of a hydra with a fine needle until it came in contact with the bottom of the dish. The hydras turned and inverted in this manner fell into three distinct classes: 1) those not able to adjust themselves to the new situation, 2) those which attempted to return themselves and 3) those which remain turned and regenerated. This last class did not recover but regained a normal organization by the rearrangement of the cells constituting the ectoderm and endoderm. The regeneration was effected by the migration of the cells constituting the ectoderm and endoderm in opposite directions through and beyond the mesoglea. Sections of hydra fixed in corrosive acetic and stained _in toto_ with borax carmine, two hours after

turning, showed the ectoderm and endoderm of the trunk in practically normal positions. No explanation was offered by Roudabush for the way in which the cell migration occurred.

During this work on regeneration it was noted by Roudabush (1934) that, during the process of turning the hydra inside out, a number of individuals were torn at the anterior end. These were isolated in culture dishes and examined at intervals to see what the outcome would be. A large number regenerated into normal individuals while a smaller percentage was found to develop two anterior ends and eventually the division was completed. It was judged that these animals underwent a longitudinal division, which was caused by an external stimulus, and that the process of division therefore, was one of regeneration rather than reproduction.

Papenfuss and Bokenham (1939) sought to find out if Hydra could be regenerated from either the ectodermal or endodermal layer alone. When entire cell layers were cultured no regeneration occurred; instead the tissue disintegrated. That the cell layers did not develop was due to the fact that the differentiated cells of one tissue layer were not able to transform into the cell types characteristic of the lacking tissue layer, and the undifferentiated cells did not become activated to regenerate this tissue.

DISSOCIATION. In an attempt to find out if isolated cells of Hydra would reunite to form a complete individual, Papenfuss (1934) obtained dissociated cells of the green hydra, Hydra viridissima, and a brown hydra (not clearly defined

taxonomically), by using Wilson's method for the dissociation of sponges. Hydra were expressed through silk bolting cloth of about forty micra, and the expressed tissue cultured in spring water. The precipitate contained free ectodermal cells, free entodermal cells, and groups of either ectodermal or endodermal cells. The groups of cells contained interstitial cells located at the bases of the ectodermal cells. In no case were observable fusion masses formed. The cells did not unite and no regeneration occurred. This failure was probably due to conditions resulting from the dissociation. As was mentioned, the expressed tissue contained groups of either ectodermal or endodermal cells, but rarely both kinds attached together. Fragments cut to contain ectoderm and endoderm united by the mesogleal layer did fuse, and eventually hydras regenerated from them. A striking characteristic of the small fragments was the amoeboid movement of the endodermal cells. The fragments came together as a result of revolving movements on the part of each fragment. The fusion was not one of attraction, for the fragments were observed by Papenfuss to break apart again. When two fragments came in contact a clear hyaline process from one fragment extended over a similar process from the other fragment. Other processes were sent out and soon they became interlaced. Fragments first united to form a plate with ectoderm on one side, endoderm on the other. The edges rolled together to form a cylinder, the endoderm being within. After the body was formed the head and foot

were produced. Sometimes irregularities and abnormalities were produced in the early stages of regeneration, probably as the result of incomplete fusion in some of the regions. Therefore, Papenfuss concluded that in order to form fusion masses there must be pieces containing both ectodermal and endodermal cells held together by the mesogleal layer as in the normal specimen.

TRANSFORMATION. Regeneration in some of the lower animals consists of a transformation of a piece into a new animal of smaller size, the transformation being brought about by a change in the form of the piece itself rather than through the production of new material at the cut ends. If a ring was cut from the body of a hydra, Morgan (1901) found that the open ends of the ring soon closed by contraction of the sides of the piece and in a few hours the ring became a hollow sphere. After a day or two the piece elongated and tentacles appeared. This was not the replacement of missing parts, but rather the transformation of the old piece into a new organism.

ANTERIOR-POSTERIOR REGENERATION. King (1901) found, that by splitting the oral end of specimens of Hydra viridis double headed hydras were produced. If the head were cut off and the body then split longitudinally through the oral end, the total number of tentacles produced on the double headed forms was greater than the original number of tentacles by an average of three and four tenth's per hydra. After a time the double headed forms separated, forming two complete

individuals. When the cut edges united after longitudinal splitting, there was an increase in the number of tentacles due to the rapid formation of tissue at the region of injury.

When the aboral end was split longitudinally a double footed hydra was produced.

LATERAL REGENERATION. If a triangular piece was cut from the side of a hydra, Morgan (1901) found that the wound healed without the formation of a new structure at the place of injury. From this it would appear that hydra is incapable of lateral regeneration.

OBLIQUE REGENERATION. When oblique cuts were made near the head to the foot region, small lateral pieces were produced. According to King (1901), sometimes these pieces were small and were resorbed, but if these pieces were large, the polyp developed a head which never had as many tentacles as the original. This later separated and formed a complete individual. When the cut was reversed, that is, from the foot to the head region the same result was produced.

GRAFTS. In grafting two polyps by their aboral surfaces after the removal of the foot ends, then after fusion has taken place cutting each component of the graft close to the line of union, King (1901) found a head formed on the oral surface and a foot on the other so that a normal individual was produced. If transverse cuts were made a head developed on each of the exposed oral surfaces and the two components of the graft pulled apart.

PHYSIOLOGICAL GRADIENTS. Physiological regeneration is not sharply separated from that including cases of regeneration after injury or loss of a part. Both processes appear to involve the same factors. Weimer (1934) showed that in hydra the distal fragments in the mass determined where the hydranth, which is the nutritive zooid, formed.

By cutting hydras as shown in Figure 2, page 22, Weimer (1932) classified the regenerated types according to their polarity as unipolar, bipolar, or multipolar. Unipolar forms are those which have one main axis of polarity. Bipolar forms are described as those forms having a complete hydranth at each end, or a complete hydranth at one end with an incomplete hydranth at the other represented by one tentacle. Multipolar forms are those in which the buds appear at the proximal level of the axis followed by stalk differentiation.

To determine whether regions of the body other than the peristome would function as organization centers, and if a differential of contact would have any effect on such organization centers and reconstitution of the masses, Weimer (1934) sectioned bodies of Hydra oligactis, then centrifuged the pieces. When first removed from the centrifuge tube, the masses had the appearance of mosaics. Three to four days later small rounded elevations appeared, which later became hypostomes, and near these were knoblike tentacles. Five to six days after centrifuging the masses were well developed. He concluded that polarities, if determined, should result in a high frequency of multipolar forms since the aggregates

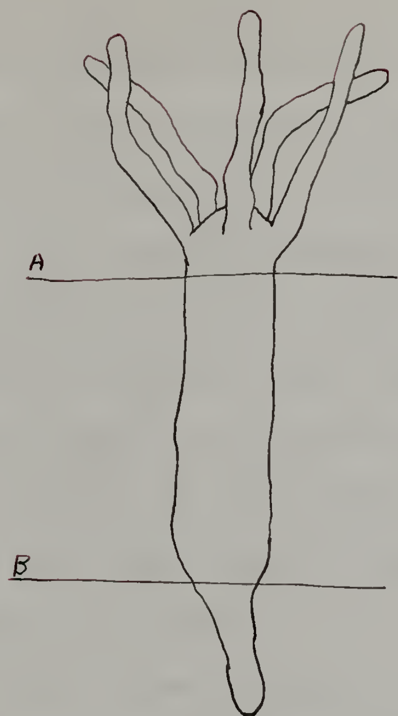

Figure 2.

Diagram of cuts made to obtain polarity in the hydra. (Weimer 1932)

would contain more than the cell group, which would become the organizer of a new apical region. Apparently this happened for from his results, all the aggregates were either bipolar or multipolar, the multipolar forms predominating. He concluded also that the determination and organization of new polarities was brought about by pieces, with a high rate of metabolism, from the body regions. The pieces were incorporated in the mass and retained the high rate of

physiological activity, because observations on the free or exposed surface, when compared to the contact or lower surface, showed more developing apical structures or new polarities. The first well developed apical region to appear on the mass seemed to determine a primary gradient.

Unipolar forms when fed, Weimer found, grew until the hydranth and body region had attained their normal size. Then four to five millimeter buds began to appear first on the most proximal end of the animal in what would be the budding zone if a stalk and foot were present. The average length of time from sectioning to when the buds appeared was twenty to twenty-five days.

Bipolar forms, after being fed, were found by Weimer to produce buds twenty-eight to thirty-five days after sectioning. The buds appeared in the region equidistant from the apical region. These developed normally and separated from the parent. Two or three days later there was a constriction in the region adjacent to the buds and the first indication of a stalk appears. Sixty to seventy days after cutting there was the first indication of a foot. Seventy to seventy-five days after cutting the individuals separated.

SIZE. Pieces must be of a certain size to regenerate. Peebles (1897) found that pieces of hydra measuring less than one-sixth of a millimeter in diameter, which is equivalent to one two-hundredth of the volume of a hydra, did not regenerate. Failure to regenerate was due to the absence of

sufficient material necessary for the production of the typical form.

King (1901) found that the size of the hydra was one of the important factors in determining the number of tentacles which would regenerate on the hypostome of a new individual formed from a piece of the body wall. Fewer tentacles regenerated than were removed. Those individuals, which had originally the largest number of tentacles, regenerated the greatest average number. There was considerable individual variation in the size of the hypostomes, but in general the size of the hypostome was directly proportional to the number of tentacles. If a polyp was regenerated from the posterior part of hydra, the hypostome decreased in size, but was in proportion to the number of tentacles borne by the new individual. If a polyp was remodelled from the anterior piece, the hypostome decreased in size to correspond with the size of the new tentacles although there was no decrease in the number of tentacles.

<u>TEMPERATURE</u>. In Hydra viridis, Peebles (1897) found that regeneration will take place at an optimum temperature of twenty-six to twenty-seven degrees Centigrade in forty-eight hours. At lower temperatures the regeneration was much slower. At twelve degrees Centigrade regeneration took from ninety-six to one hundred sixty-eight hours to be completed.

<u>FOOD</u>. Weimer (1932) found that in animals from which the hypostome and tentacles had been removed the pieces had

developed at least one perfect apical region bearing a hypostome and tentacles by the third or fourth day. It was then possible for the animal to begin feeding.

TUBULARIA.

Tubularia are coelenterates having large solitary or colonial polyps which are pinkish in color. The hydranths bear a basal and distal whorl of filiform tentacles.

The cutting of a hydroid stem has long served as a stimulus for the regeneration of a hydranth, but the exact nature of the stimulus is not known. Cutting off a part does two things according to Barth (1940). 1) It releases cells at the cut surfaces from physical and chemical influences of the part removed. 2) It creates a new external environment. A number of factors are also involved. 1) Some cells at the cut surface may be injured giving rise to wound hormones which stimulate growth and differentiation. 2) The exposed cells are able to lose substances more readily. These may possibly be inhibitory substances which prevent regeneration. 3) The exposed cells are in direct contact with oxygen in the medium. 4) The cells are exposed to the direct action of salt in the solution.

Morgan (1906) described the stimulus for the formation of the polyp as being an internal one. The stimulating agent, he stated, was a material set free either at the ends or by the entire walls of the stem and was soluble and able to pass into

circulation.

It has been well established that any group of cells of Tubularia, if large enough, will organize itself into a hydranth. Early workers showed that sections of a stem cut at any level would regenerate.

The removal of the hydranth in Tubularia is not sufficient to cause regeneration, since, if a ligature is tied through the stem after cutting off a hydranth, regeneration is inhibited. Morgan (1906) found that, when an oral and a basal end of halves of the same piece were exposed at the same level, the cut oral end develops hydranths before the cut basal end. By tying the oral end of the piece, then as soon as the basal hydranth had begun to develop, by cutting off the oral end of the piece below the ligature, the development of the oral hydranth was delayed or suppressed.

OXYGEN. Oxygen is an important factor in regeneration of Tubularia. The mechanism for the formation of a hydranth from a given region of the stem depends to a great extent upon the oxygen supply. The tissue of the stem will not form a hydranth within the perisarc because the oxygen supply may be too low, or the oxygen comsumption may be too low for the process to occur. Any treatment that will increase the oxygen consumption (removal of the perisarc, injection of oxygen gas, or even shaking) will start regeneration. The hydranth forms in relation to the increased oxygen supply, with the oral end of the regenerant at the point of the highest oxygen tension.

Morgan (1903) observed that pieces of Tubularia stems, which ordinarily regenerated hydranths at both ends when cut, formed single hydranths at only one end, the free end, when the other was set upright in the sand. He deduced that oxygen in the sea water aided in regeneration.

Miller (1937) found that when one end of a stem was bathed in oxygen in sea water and the other in boiled sea water, hydranths developed on the end bathed in oxygen. Circulating sea water produced more hydranths than did standing sea water. When nitrogen was bubbled through the sea water there was complete inhibition at the end bathed. Barth (1937) inserted distal ends of Tubularia into a glass tube and found inhibition of regeneration at that end with an increase in regeneration at the opposite end. He attributed the inhibited regeneration to the low oxygen rate.

Since hydranths do not develop at a cut surface when the perisarc is covering the surface, it has been supposed that the perisarc interferes with the circulation of oxygen to the cut surface.

REMOVAL OF THE PERISARC. Zwilling (1939) demonstrated that in Tubularia exposure of the stem without cutting was sufficient to cause regeneration. When a portion of the perisarc covering was removed from the stem the coenosarc appeared as rather indeterminate masses of material. There seemed to be, according to Zwilling, a competition between the tendency to retain the vegetative state and the tendency to form hydranth material. The first stage in the regeneration process

was described as the appearance of the pink pigment. Depending on existing conditions, the coenosarc became either definitely determined to form hydranth tissue or retained it's original condition. A hydranth formed, broke through, and a thin perisarc was secreted in the early stages. If it remained in it's original condition the pink pigment disappeared and the coenosarc continued to produce more perisarc, but no hydranth was formed.

There was found to be a variation in the morphology and polarity of the regenerant, which depended roughly on the amount of coenosarc tissue exposed. When the exposed area was small, only a limited amount of coenosarc immediately surrounding the area was involved in regeneration. The tissue did not extend completely around the stem but formed a curved disc. When the proximal tentacle buds appeared they radiated out from the exposed central area. These regenerants emerged as complete single hydranths whose polarity (oral-basal axis) was at right angles to the original polarity of the stem. When large areas were exposed a complete cylinder of coenosarc was involved in the hydranth formation. Tentacle buds were formed symmetrically around the stem and when two hydranths emerged they were complete and mirrored images of each other. They remained fused at the hypostomal region. The original polarity was retained in the proximal regenerants, and completely reversed in the distal. Zwilling explained that the coenosarc must be metabolizing at a certain level before the hydranth can be formed. When a small opening was

made in the perisarc, only the tissues immediately around the opening got enough oxygen to raise them to this level. Since the basal portions of a hydranth would form at a lower level of activity, symmetrical around the stem, a small complete hydranth was formed. As more oxygen was admitted to the stem, more coenosarc was activated to form hydranth tissue.

The stimulus to regenerate was not one of cutting or injuring the tissue, but exposing the uncut stem to sea water. This can mean that either some inhibitory substance escaped from the free surface or that an increased supply of oxygen stimulated the stem to regenerate. Rose (1940) found that oxygen gas injected into the coelenteron caused regeneration in the stem. In some of the stems the regeneration occurred at the distal end even though the perisarc was tied over that end. When injected, oxygen breaks up into a number of small bubbles and a region of regeneration is set up about each bubble. If the stem was cut at these regions a hydranth formed. Since there was no escape for the inhibitory substances, the sole factor in stimulating the regeneration was the introduction of oxygen.

Miller (1942) found that there was evidence of inhibitory substances which must be allowed to escape from the stem. The fact that carbon dioxide combined with water to produce carbonic acid would suggest that the inhibitory action of the gas might result in an increased acidity of the solution. He found there actually was an increase in the acidity of the contents of the coelenteron of stems enclosed in tubes.

However, the oxygen content of the coelenteron was not affected by an increase in that of the external environment.

Thus it is interpreted that the regeneration of Tubularia is dependent upon the amount of oxygen the tissues receive. Since the perisarc is impermeable to oxygen, the stimulus for the regeneration is the admission of oxygen due to the cutting of the perisarc.

RATE OF REGENERATION. The physiological gradient in the stem is exhibited by the graded rates of regeneration at various levels of the stem. The rate of regeneration is the measure of the chemical changes involved in the differentiation of the hydranth from the stem after cutting. By utilizing the variables of size and time, Barth (1938) stated it was possible to express the rate of change within the stem at any level and thus compare rates under various conditions. He measured the rate of regeneration by the formula $R=\pi r^2 L/t$, where r is the radius of the cross section, L is the length of the regenerate and t is the time in hours for the emergence of the fully formed hydranth.

Hyman (1926) had found that level was an important factor in determining the dimensions of regenerated oral hydranths.

Barth (1938) found that the rate of regeneration of isolated parts of the stem decreased from the distal to the more proximal regions. Barth showed that an increase in the length of the stem adjacent to the regenerating end increased the rate of regeneration. If the stem was short the distal end became dominant and inhibited the proximal end. Hyman

(1920, 1926) stated there was evidence of a metabolic gradient in the rate of regeneration with respect to level. The amount of tissue regenerated in a given time appeared to be dependent upon a metabolic rate. The more apical the level within the limits of the primary gradient the more rapidly does it produce an apical end. She also found that the axial differences in the rate of regeneration are independent of size or mass differences at different levels. The more apical the level from which the piece was taken the larger was the size of the oral (apical) end regenerated and there was a greater mass of regenerating tissue.

There is a difference in the rate of regeneration of apical and basal pieces which exists under normal conditions. This difference can be reduced by using stems bearing branches and cutting the apical piece above the branch and the basal piece below the branch. Since the first branch marks the limit of Tubularia, individual apical pieces above such are really basal and the basal pieces below the branch are near the apical end of the second individual. The difference in the time of regeneration is less when pieces are cut from a corresponding level of the stem without branches.

POLARITY. Attempts to analyze the role of the environmental factors in regeneration and the origin of polarity in Tubularia are complicated by the heterogeneous nature of the stem. By polarity in Tubularia is meant the formation of a hydranth on the oral end and a stolon on the basal end. Some of the factors contributing to the polarity of the regenerant

are the gradient in the rate of regeneration and the gradient of oxygen consumption of the stem. It has also been proven that the distal hydranth exerts a dominance over the proximal cut end and the latter will not form a new hydranth unless the distal hydranth is removed. Child (1927) also found that dominance controlled lateral budding.

Morgan (1904) tried to explain polarity on the basis that the oral polyp, which develops first, needs the nutritive material for it's development. This it finds in the coenosarc or in circulation and uses it in it's development. Consequently the cut surface at the basal end cannot get material necessary for it to develop into a hydranth, and remains undeveloped or produces a stolon.

It has been found by later investigators (Miller, 1937, Zwilling, 1939, Goldin and Barth, 1941, and Goldin, 1942) that when the coenosarc is removed from the perisarc a series of reorganizational changes occur involving morphological and physiological dedifferentiation. The exposure of the entire coenosarc to sea water, it was thought, might result in sufficient reorganiaation to obliterate the existing gradients in the stem. Goldin and Barth (1941) classified regenerated coenosarc fragments as, unipolar, bipolar, bipolar-unipolar, multipolar, and apolar. Unipolar are described as those coenosarc fragments which regenerated a single hydranth on the rounded mass of tissue. Bipolar forms regenerated two hydranths at two opposite poles of the coenosarc. Bipolar-unipolar forms regenerated two hydranths from the same region of

the coenosarc fragments. Multipolar regenerants are those having more than two hydranths. Apolar are those forms which did not regenerate.

The greatest percentage of coenosarc fragments regenerated unipolar forms. Multipolar forms constituted a much smaller percentage. Since exposure of the naked coenosarc to sea water is a sufficient stimulus for hydranth formation, the formation of hydranths should be enhanced when the naked coenosarc is in contact with sea water and the oxygen dissolved in it. That this is true was shown by the high percentage of multipolar forms obtained by Goldin and Barth. The high percentage of unipolar forms may have been due to the exposure of a uniform gradient of oxygen in the sea water, they suggested. That the initial polarity was lost was shown by the appearance of regenerated hydranths at the free surface of fragments which had become attached, the regenerated hydranths having no relation to the original polarity.

Goldin (1942) found that polarity could be determined by different exposures of the coenosarc. Fragments were placed inside glass capillaries so that half the surface of the fragment was covered by the capillary, the other half exposed to sea water. Of fragments thus experimented upon, new hydranths appeared at the exposed surfaces, none on the capped surfaces. The regenerated fragments were unipolar, and polarized in the direction of the open end of the capillary. Since the coenosarc fragments at twenty-four hours after removal from the perisarc have lost their original polarity, the

polarity of the regenerant is new and is developed in response to an imposed environmental differential.

DOMINANCE. The phenomenon of dominance has been a subject of much discussion. Hyman(1920) found that the differences in electrical potential along the axes form a manifestation of metabolic gradients. She found the difference of electrical potential between a hydranth and the distal regions of the stem was greater in the case of larger hydranths and less in the case of smaller hydranths. Also the hydranths were more negative to distal portions of the stem than to the proximal regions where lateral branches were present. However, the proximal regions were more negative to the distal especially when they bore branches. The potential difference between distal and proximal regions of the stem was always less than that between the hydranth and the distal regions of the stem.

Barth (1934b) found that the electrical potential differences between regions of the Tubularia stem varied. In another paper (1934a) he found that polarity could be reversed by electric current, and there were varied results in the inhibiting effects of the electric current.

Barth (1938) stated that dominance may be exerted through the transportation of substances. Tubularia shows a gradient of inherent rates. Therefore there must be graded differences in the concentration of some substances which account for the different rates. Barth used the symbol "E" to represent these substances, and found "E" was present in highest concentration

in young cells at the distal end. It's lowest concentration was at the proximal end. Therefore, he explained, internal factors were responsible for the differing rates at various levels of the stem. Another factor travelling in the stem and causing an increase in rate was called "S". This factor was transported through the gastrovascular cavity in the circulation. If one end was using up the materials in rapid regeneration the concentration of "S" in the circulation would be lowered, so at the opposite end substances might pass into the gastrovascular cavity from the cells and inhibit regeneration by the removal of available materials. This process was essentially one of chemical change. When the circulation "S" was blocked by a droplet of oil injected into the coelenteron the dominance was blocked. Both the distal and proximal ends regenerated independently of each other and no dominance was exerted by the distal end.

TEMPERATURE. Recent work by Moog (1942) and Moore (1939) suggests that the critical point for regeneration in Tubularia approximates twenty degrees Centigrade.

X-RAY. Curtis and Ritter (1927) subjected Tubularia to x-radiation and found no regeneration of the removed hydranths occurred, although the coenosarc remained alive as was shown by the active circulation of particles within the enteron.

STRYCHNINE. Miller and Miller (1937) found that continuous exposure to strychnine resulted in a decrease in the size of the hydranth primordia and in an increase in the time of development. A definite depression of the process of

regeneration was noted.

PENNARIA.

Pennaria is a branching colony found in shallow water. The hydranths bear a basal whorl of filiform tentacles and also short knobbed tentacles on the hypostome.

DISSOCIATION. To obtain dissociated cells in Pennaria tiarella, Wilson (1911) cut the hydroid into fragments and pressed them through silk bolting cloth, after his method of obtaining dissociated cells in sponges. He observed fusion to take place at once, and within a few hours the tissue had formed reticular sheets on the bottom of the dish. Within a day, the fusion masses were surrounded by a distinct perisarc which was pink. About three days after pressing, outgrowths were formed, the original mass being spheroidal with a thick perisarc. Wilson described these outgrowths as adhering at first to the bottom, later rising, and having on their extremities reddish-brown knobs resting on lighter colored stalks. Six days after pressing the outgrowths bore hydranths, each with filamentous tentacles and upper short capitate tentacles.

When the coenosarc (stem material) was pressed through gauze in the same manner by Wilson, thus breaking pure coenosarcal tissue into cells, he stated that fusion occurred in twenty minutes. On the next day a distinct perisarc was observed. On the third day, outgrowths had formed and later these transformed into hydranths bearing short tentacles.

HISTOLOGICAL STUDIES. In a drop of live tissue pressed through gauze and kept under observation, small masses formed, according to Wilson, which were inclusive, having endoderm cells, cnidoblasts, and pale cells probably of ectodermal origin.

In studies of the fusion masses sectioned and stained, Wilson showed the masses to be solid bodies of uniform structure with no stratification into layers. Ectoderm and endoderm layers had begun to differentiate and surround a central cavity.

In the pressed out material fixed and stained, Wilson saw quantities of large granular cells, many of which were spheroidal. These he believed to be endodermal granular cells. There were also droplets of a translucent substance varying in size yet smaller than the endodermal cells, which were common. This material was interpreted to represent minute fragments of protoplasm that had rounded off. The ectoderm was composed of finely granular material and sometimes included nettle cells.

REMOVAL OF HYDRANTHS. Another method by which regeneration was brought about in Pennaria was by the amputation of the hydranths from young branches on the colony, as investigated by Puckett (1936). If pieces were laid flat on the bottom of the dish the coenosarc, on emergence from the perisarc, came in contact with the dish and holdfasts, instead of hydranths, were regenerated. So the branches were suspended in running sea water, and only hydranths were regenerated, even at the basal end of the stem.

Morphologically, Puckett observed the process of regeneration as consisting of wound closure which took place about six hours after amputation, and appeared to be due to an approximation of the cut edges of the coenosarc. The regeneration processes then set in and in another six hours there was the first indication of a regenerating hydranth in the formation of a knob like protuberance by the coenosarc. This was covered by a thin membrane layer of perisarc which was ruptured when the new hydranth emerged. The coenosarc bud continued to grow and tentacles appeared as small evaginated buds on the body of the hydranth about twenty-four hours after amputation. The proboscis appeared as an evagination from the distal end of the bud, and late in the process the mouth opening broke through. Regeneration was completed within thirty-six to forty-eight hours after amputation of the hydranths.

X-RADIATION. Investigators have demonstrated that x-radiation is a valuable tool in studying the process of regeneration in various animal groups. By means of x-ray, abnormal conditions have been produced in the tissues and cells of normally regenerating structures which result in their failure to regenerate.

To determine the effects of x-radiation, hydranths were removed and the branches immediately radiated in small dishes of sea water. Puckett (1936) found that dosages of ten thousand five hundred Roentgen units or larger completely inhibited the regeneration of hydranths. Dosages of a lesser amount retarded regeneration of hydranths, but did not inhibit their

formation completely.

A formation of fresh tissue took place during the first twelve to eighteen hours after radiation at the cut surface of the branch, forming rounded buds which were never able to differentiate into new hydranths.

Since in Pennaria, as in other hydroids, the individual hydranths are connected by a common coenosarc and share a common circulation by means of a coelenteron, Puckett investigated the effect of x-radiation on the regenerative abilities of a colony. To find out if there are any toxic substances which may be formed in the exposed portion and which may pass over to the unexposed portion to retard or inhibit hydranth regeneration, a portion of the colony was shielded from radiation by a lead plate. The experimental set-up used by Puckett is shown on page 40, figure 3. One completely screened colony, C, and one completely exposed colony, A, were used as controls. All the hydranths had been amputated and the branches were arranged in such a way that one branch was exposed to radiation while a remaining connected portion was protected by a lead plate. A single exposure to x-radiation of ten thousand five hundred Roentgen units was given.

Puckett found that the colony exposed to radiation (A) failed to regenerate the lost hydranths. The completely screened colony regenerated lost hydranths within forty-eight hours. In the partially exposed colony (B) the portion protected by the screen from the radiation regenerated it's lost hydranths in forty-eight hours.

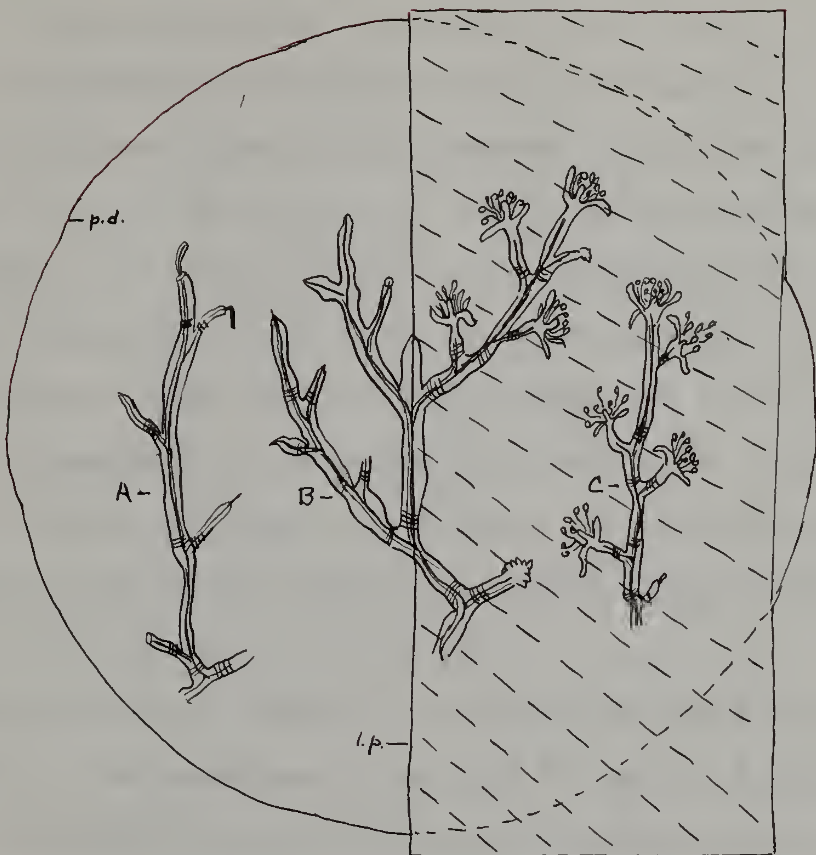

Figure 3.

Diagram of x-radiation experiment.
(After Puckett).

Colony A radiated control

Colony C screened control

Colony B x-radiated portion

p.d. petri dish

l.p. lead plate

The idea that a toxic substance is formed in the radiated portion which may spread throughout the colony is discredited, for the non-regenerating branches of the radiated side acted as if independent of the unradiated tissue, and there has been no evidence found of any regenerating promoting substance passing from the unradiated to the radiated portion.

SALTS. In certain marine invertebrates moderate dilutions of sea water have been shown to increase the rate of regeneration. Kiel (1932) investigated the effect of salts upon the regeneration of Pennaria. Solutions of glycine, urea, glycerin, glucose, and lactose of the same osmotic pressure as sea water were used. These proved toxic to Pennaria and no regeneration occurred.

In determining whether the nature of the ions influenced the rate of regeneration, solutions of sodium ions, calcium ions, potassium ions, and magnesium ions were used in the same concentrations as in sea water. In no case was there an acceleration in the length of regeneration, but rather a retardation. Salts also tended to retard the rate of regeneration in Pennaria.

LIGHT. Loeb (1891) and Goldfarb (1906) have found that the regeneration of hydranths of Pennaria takes place when the animal is exposed to light. If the hydranths were removed from a colony, and the colony kept in the dark, hydranths did not appear; but if the colony were brought back into the light the hydranths would appear. Loeb tried the effects of different colored lights on regenerating hydranths. Colonies kept

under blue glass plate regenerated new hydranths on the fourth day. Not a hydranth appeared on the colonies kept under red glass. On the ninth day the red glass was replaced by the blue, and the hydranths began to appear. He explained this by assuming the refrangible (red) rays acted as darkness. Goldfarb found that exposure to diffuse light varying from a few minutes to nineteen hours did not result in hydranth formation, but exposure to brilliant light for not less than two days regenerated hydranths. Pennaria colonies kept in the dark never developed hydranths, therefore, light is essential for regeneration in Pennaria.

THYROXIN. Torrey (1934) found that regeneration of the hydranth in Pennaria was depressed by thyroxin solutions in a concentration of one part to fifty thousand. He also found that metallic iodine was a more effective depressant on regeneration than the thyroxin.

ELECTRIC CURRENT. Barth (1934a) obtained anodal inhibition which was complete at sixty-two volts. This inhibition was found to be reversible.

When the hydranth was placed at right angles to the current, the hydranth bent towards the anode and the tentacles contracted on the anodal side of the hydranth.

He concluded that there was no coincidence of electrical polarity and organic polarity. That during regeneration a bioelectric current may flow externally either to or from the actively regenerating region was shown in a later paper (1934b) Any interference with the bioelectric current retarded

regeneration.

EUDENDRIUM.

Eudendrium is a branching, colonial hydroid which has a distinct and annulated perisarc. The hydranth bears a single whorl of filiform tentacles.

DISSOCIATION. To obtain reconstitution from dissociated cells of Eudendrium ramosum, the hydroid was pressed through silk gauze in the manner described previously. It was found by Wilson (1911) that masses fused almost immediately. Four days after dissociation an outgrowth occurred with a definite perisarc covering. A day later he observed clearly the ectoderm and endoderm. A few days later, an outgrowth adhered to the glass, like a hydrorhiza, and another projected upward into the water, beginning to take the form of a hydranth. Later the ectoderm contracted away from the perisarc, and nettle cells were observed by Wilson on enlarged tentacular ends.

Wilson made sections of the restitution mass when it was twenty-four hours old. These studies showed the perisarc surrounding the living tissue. In the interior of the mass cnidoblast cells were common. Between the cells, protoplasm existed as a vague reticulum with scattered nuclei in it.

STIMULUS. Goldfarb(1907) found that in the regeneration of Eudendrium from which the hydranths were removed, any severe injury at any level of the colony may cause polyps to regenerate if they are exposed to sea water. This was also found

to be true for Tubularia. The maximum number of polyps regenerated by Eudendrium was found not to occur in normal sea water, but in a solution of sea water diluted with twenty per-cent tap water. When the hydranths and the primary branches were removed and only the main stem of the colony was kept in sea water, the new hydranths were regenerated in about forty-eight hours.

TEMPERATURE. Goldfarb also found that regeneration in the number of hydranths increased with an increase in temperature to the optimum. Regeneration increased up to twenty-eight degrees Centigrade. With a lowering of temperature, the hydranth regeneration was decreased and at ten degrees Centigrade regeneration was inhibited.

CONTACT AND PRESSURE. Contact, pressure, and impact were found to be inhibiting influences which tended to prevent complete development at those ends which came in contact with or are pressed upon a solid body.

LIGHT. The hydranths were removed by Goldfarb (1906) in a dark chamber and the colonies kept in sea water in the chamber. About forty-eight hours after removal of the hydranths, new hydranths were formed appearing as single hydranths or groups of hydranths.

Direct rays of the sun were injurious as no polyps developed.

Colonies kept in the dark and then exposed for brief periods were stimulated to regenerate more readily than those kept in the light.

Goldfarb concluded that darkness does not prevent the regenerative processes in Eudendrium from taking place, nor does it retard nor decrease the number of hydranths formed.

ELECTRIC CURRENTS. Barth (1934) in determining the effect of electric currents on regeneration found no cathodal outgrowths. The anodal outgrowths are both basal and apical, and hydranths form from some of them. He also found that when the hydranth was placed at right angles to the current, the hydranth bent toward the negative cathode pole. He concluded that this was due to contraction, since the hydranth returned to the original position when the current was broken. The tentacles contracted on the cathode side when the hydranths were placed at right angles to the current.

INTERNAL FACTORS. Goldfarb (1907) found that within the perisarc the coenosarc can move en masse, and invariably moves toward the basal end of the piece. He observed that regeneration occurs only where the coenosarc is present and stated that whether regeneration shall or shall not take place at a a cut end is determined by the migration of the coenosarc. This crowds the coenosarc at the basal end of a piece, and if conditions are favorable a polyp readily appears there. He also stated that the migration of the coenosarc is furthered by inverting the pieces, which is almost certain to stimulate regeneration at the basal ends.

Internal factors affecting the regeneration of Eudendrium can be age, which determines the rate of regeneration and also the number of hydranths regenerated, and the amount of

coenosarcal influence on the number and position of the regenerating polyps.

REGENERATION IN OTHER HYDROIDS.

PODOCORYNE. Hargitt (1915) found that by pressing cells of Podocoryne carnea, a hydroid on Limulus, shells of hermit crabs, and rocks, the precipitate yielded cells of ectoderm, endoderm, nematocysts, and interstitial cells. The cell aggregates were sub-spherical in shape and showed considerable variation, resembling embryonic blastulae or morulae. After aggregation there was a process of encystment consisting of the secretion of perisarc about the entire mass and the adhesion of the mass to the bottom of the glass. Hargitt described hydranth formation during the second day as being accomplished by the protrusion of bud like growths through the perisarc. On the fifth day after dissociation of the hydroid cells, a fully formed hydranth was formed, bearing a hypostome and three tentacles. Full development occurred within the next two days.

Hargitt also found that the rate of development differed among the different fusion masses. Small individuals were usually developed from small aggregates.

HYDRACTINIA. Hydractinia echinata is a polymorphic, unbranched, colonial hydroid which exhibits under certain conditions a change of polyp form on regeneration.

Wilde (1940) found that if pieces of the stalks of the gonozooids (the reproductive individuals) were severed from

the living substrate and cut below the sporosacs, regeneration occurred in all cases and the gonozooid form was reconstituted.

Gastrozooids cut in the same manner by Wilde below the tentacular ring also regenerated, the result being that in all cases well developed gastrozooids were formed. When small pieces of the sporosac region of the gonozooids, stained with Nile blue sulphate, were grafted into the oral tissues of the gastrozooids below the tentacles, most of the pieces regenerated and formed either a gonozooid or a gonozooid and a gastrozooid.

Wilde believes there are definite influences in certain regions of the polyp during regeneration. The gonozooid influencing region is in the most oral third of the gonozooid. The horny-covered living substrate is the gastrozooid influencing region. He concludes that there is a balance between the two regions, that which is dominant being expressed in the final form of the regenerate.

<u>GONIONEMUS</u>. An unusual process of regeneration is that of incomplete regeneration. Hargitt (1902) found that in Gonionemus vertens, the cut edges came together, fused, and the pieces assumed the form of the bell, but the missing parts were not replaced. Morgan (1901) found that in an individual cut in two, shown by figure 4, page 48, each half closed in and became bell shaped.

Each new individual had only two of the original radial canals that each half had when separated from the other. A faint line along the region of fusion of the pieces appeared

to represent a new radial canal, and each half proboscis had completed itself. No new tentacles formed except perhaps one or more where the cut edges met. Actually there seemed to be little regeneration, although a typical jelly-fish was assumed by each half piece.

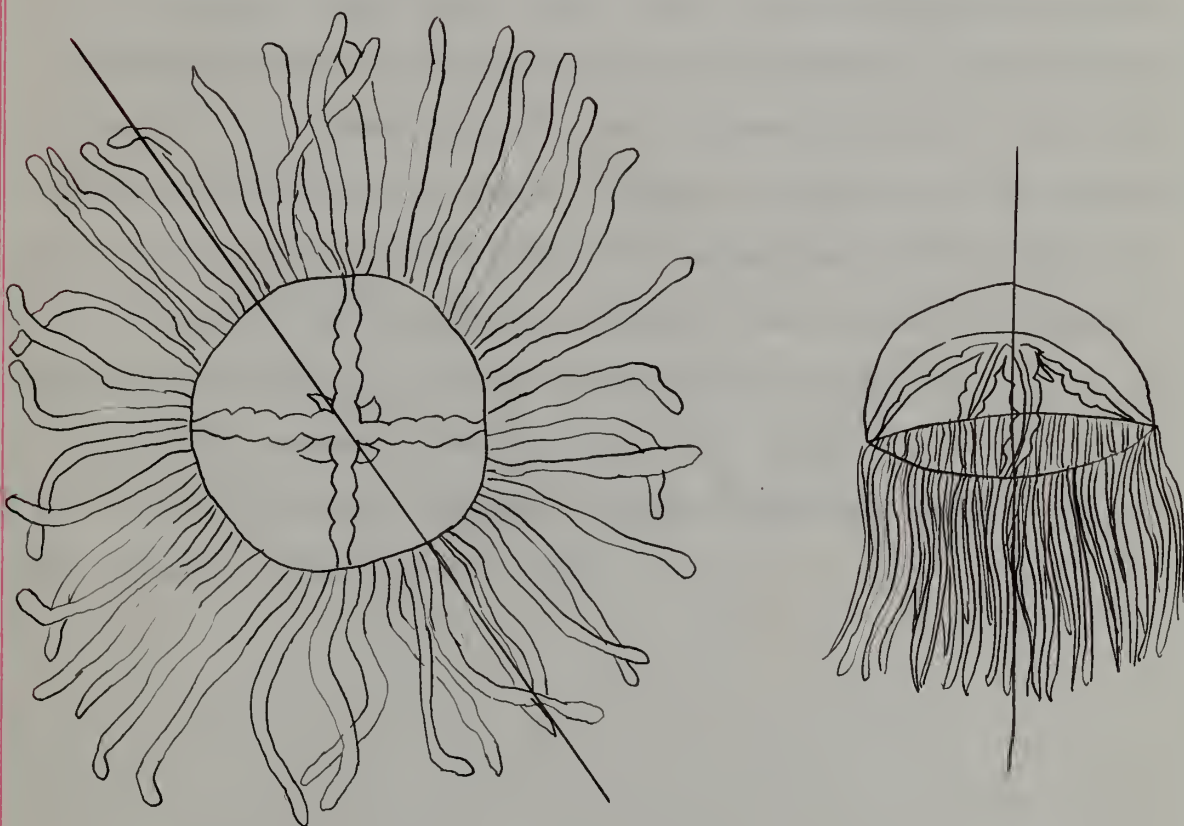

Figure 4.

(After Morgan, 1901)

Morgan also found if the jelly-fish was cut into four pieces, each piece containing one of the radial canals, these pieces assumed a ball like form. A new proboscis was developed from the proximal end of the old radial canal, and since

this end was carried to one side during the closing in of the piece, the new proboscis was lying not at the top of the sub-umbrella space, but to one side. Pieces even smaller than one fourth of the jelly-fish were found by both Hargitt and Morgan to assume the shape of the old bell and contained a part of one of the radial canals. However, they did not find that missing organs came back. That the animals do undergo a certain amount of regeneration was observed by these investigators in the formation of a new proboscis, and in certain cases by a new radial canal. Morgan stated that even tentacles may be partially regenerated, if the margin of the bell was cut off near the base of the line of the tentacles. Small knobs were found to appear along the cut edge, but the pieces died before regeneration was very far along. If the margin was cut off in one quadrant, Morgan found new tentacles may be produced along the cut edge.

PHYLUM CTENOPHORA.

The ctenophores are soft, delicate jellyfishes of a more or less spherical, pear shaped, or cylindrical body form. There are eight longitudinal bands of cilia, "combs", which are organs of locomotion. Each of these bands is composed of a series of transverse plates formed by the fusion of long cilia. The animal has an oral end at which is found the mouth leading into a stomach. At the aboral end is a slight cavity which is connected with the eight bands of cilia by four ciliated grooves, thus forming the statocyst or apical organ.

The ctenophores, until recently, have been regarded as a group lacking the powers of regeneration. However, regeneration has been observed and described in Mnemiopsis leidyi (Agassiz) by Coonfield (1936).

In bringing about regeneration, specimens were cut in several different positions to produce regenerating halves. One method was to cut the specimen across the body midway between the apical and oral ends. This type of cutting gave the following results according to Coonfield. New plates were formed between the old ones along the stretched rows at the oral end of the apical pieces within five days. Additional plates formed at the apical end of oral pieces in three days. An apical organ was formed in most oral pieces within two days. A second method of forming halves was by longitudinal cutting midway between the adesophageal plate rows. The cutting was done along the midline except in the region of the apical

organ. In this region the cut was made to one side of the apical organ in order to leave this organ in one of the halves. Of the halves retaining the apical organ, most lived and each regenerated four complete rows of plates within nine days. Of the halves which lived and did not retain the original apical organ most reformed this organ and four complete rows of plates, and several showed signs of regenerating either an apical organ or rows of plates by the end of the tenth day. The third method of cutting Mnemiopsis in order to obtain regenerating halves was by longitudinal cutting midway between the adtentacular plate rows. The cut was made to one side of the apical organ in order to leave the structure in one of the halves. Out of the pieces thus cut, most retained the apical organ and lived, each regenerating four complete rows of plates within eight days. Those which did not retain the apical organ, lived, but formed the organ and four complete rows of plates, and some failed to regenerate either an apical organ or rows of plates. In regenerating thirds the animals were divided by two cross cuts between the apical and oral ends thus dividing them into apical, middle, and oral pieces. The apical pieces survived and formed the plate rows and canal connections as well as lobes and auricles at the oral end. The middle pieces, which lived, regenerated the apical organ and canal connections in the apical zone, and canal connections as well as lobes and auricles in the oral end. Of the oral pieces each regenerated an apical organ and formed canal connections. Regeneration in these experiments was completed

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within seven days.

From these experiments, Coonfield concluded that in Mnemiopsis the apical organ is the regulating center during regeneration. The specimens which retained the apical organs regenerated the rows of plates within less time and in a higher percentage of cases than those which did not retain the organ. In all portions which did not retain the original apical organ, the structure was regenerated in all cases before the rows of plates. Regular organization was complete after the apical organ had regenerated. In some cases, certain pieces, which failed to form lost rows of plates after longitudinal cutting, rounded up and continued to live as normal animals. If the original apical organ had not been retained this structure was soon regenerated.

The sequence of recognizable phases of regeneration were described by Coonfield as wound closure, swelling, stretching, and reformation of the lost parts. Wound closure was accomplished by edges of the outer body wall moving together and fusing with each other. Wound closure was completed within five hours after the operation. The swelling and stretching of the region at the regenerating area was indicated by a stretching of the rows of plates. Stretching was completed within eighteen hours, and at this time plates on these rows had become widely separated. Plates regenerated on rows at regular intervals between the old plates within a few hours. When cuts were made to remove parts of the body containing rows of plates on the apical organ these structures were reformed.

The apical organ reformed first and the regeneration of the rows of plates followed in a few hours, and plates regenerated on the rows within three hours after the rows were complete. Following removal of sections from plate rows, the remaining ends of the rows pulled together, fused, stretched or formed solid cords which later became hollow. New plates formed in the healed region and in between the old plates which had been pulled apart by stretching.

PHYSIOLOGICAL GRADIENT. Two methods of testing for a gradient by absorption of apical organ grafts were used by Coonfield and Goldin (1937). Cuts were made at four levels as shown in figure 5, page 54. Certain organs were removed along with the aboral portion of the body. In the first method the apical organ was transported to different levels of the body. The results show that the grafts near the apical organ were absorbed at a slightly shorter time than those farther away. In the other method the apical organ of the host was removed at the time of transplantation, and in this way the possible influence of this organ on the absorption of the graft was eliminated. There was no appreciable difference in the time of absorption of the grafts at different levels in the body. There was a low percentage of grafts when the apical organ was removed, many of these grafts persisted however, but no regeneration occurred. This failure to regenerate was due to the inhibitory influence of the grafts and from this they concluded that a physiological gradient is not present in Mnemiopsis.

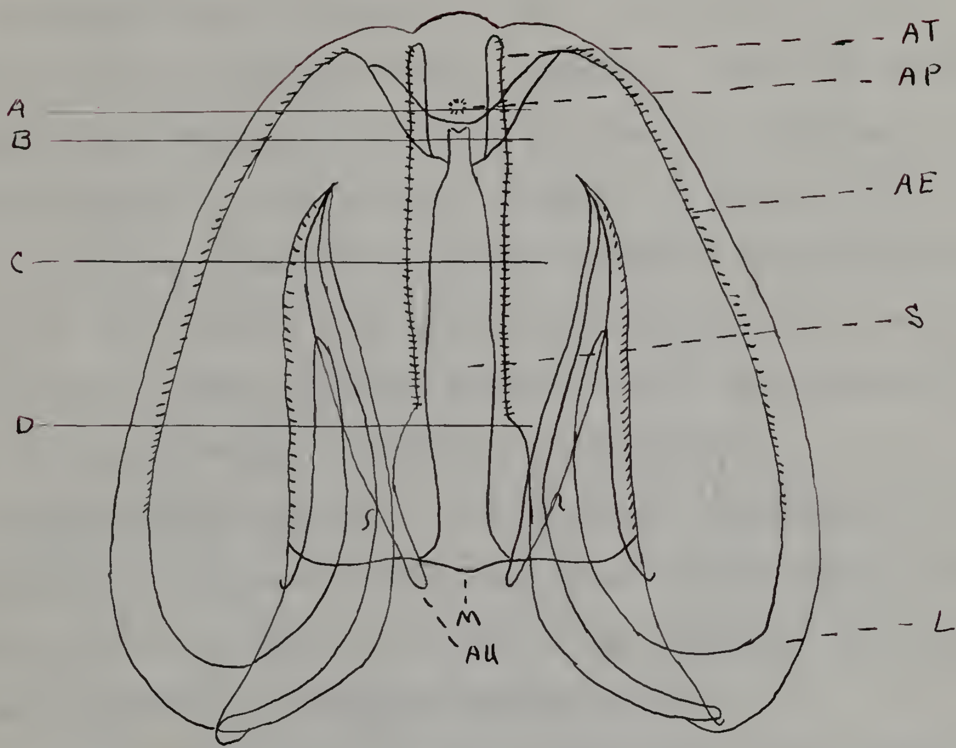

Figure 5.

Diagram showing cuts in Mnemiopsis.

AT, adtentacular row; AP, apical organ; AE adesophageal row; L, lobe; M, mouth; AU, auricle, S, stomadeum.

PHYLUM PLATYHELMINTHES.

The Platyhelminthes are the simplest multicellular animals that are bilaterally symmetrical with anterior-posterior and dorso-ventral differentiation. These animals have a digestive cavity having a single opening. They have ectoderm, endoderm and mesoderm, and also an excretory system of flame cells, opening to the outside through a pore or pores. The nervous system consists of paired cerebral ganglia forming a brain at the forward end, and nerves extending to various parts of the body. Special sense organs, when present, consist of simple eyes, tentacles or statocysts.

POLYCHOERUS CAUDATUS. Polychoerus caudatus is a small marine turbellarian which has no eyes or other sense organs and no excretory system. There is no axial gut nor central nervous system to influence regeneration.

Cut head pieces are easily stimulated into regenerative activity by changing the water or jarring the dish, while the middle and tail pieces hardly show any activity unless violently disturbed. Differences in the rate of regeneration between pieces are small.

Anterior regeneration at different levels was found by Stevens and Boring (1905) to occur not by muscular contraction and the union of muscle bands, but by a folding under and the union of the cut edges.

The histological study of regeneration in Polychoerus caudatus was described by Boring in 1905. Worms were cut into

head, middle, and tail pieces. He found that the exposed surface heals by totipotent formative cells migrating to the surface to form new tissue. The cells at the cut end secreted the cuticular substance and developed cilia. There was found a streaming of parenchyma cells toward the regenerating region.

TRICLADIDA. In the order Tricladida, belonging to the class Turbellaria, much work has been done on the family Planariidae which includes Planaria, Dendrocoelum, and Phagocata. The regenerative process by which a piece of the animal forms a perfect individual involves healing of the wound surfaces, formation of a small amount of new tissue at these regions as a result of division, localization, and differentiation of the formative cells, and regulatory changes by which the piece gradually assumes normal proportions. Even the small and irregular fragments exhibit a polarity so that in the process of growth the axes of the original body are preserved in the new individual. Each piece has the capacity to form a normal adult except in special cases.

When the cut edge is exposed there is involved a rapid covering over of the exposed tissues. This process generally takes place from the margin of the wound, and a layer of cells, usually of ectoderm, covers the surface. The closing over may be brought about by the contraction of the muscles.

In planaria it has been generally found that during regeneration, ectoderm covers the exposed surface and from it arises new ectoderm. The digestive tract appears to come in part from the old tract, and part from the middle layer of

cells. The nervous system appears to develop from the middle layer of cells found scattered through the body. These form sort of a reserve supply that gives rise to the digestive tract, nervous system, and the middle layer cells in these parts. From them arise a new pharynx and the lining of the pharynx chamber.

Curtis and Schulze (1934) have found that in regeneration there can be four possible sources of new tissue. Like cells which produced like cells; specialized cells dedifferentiated and rededifferentiated into other cell types; unspecialized or formative cells that had persisted from embryonic life might be a source; or changes might include more than one of these processes. They found that the cells of planarians do not produce others like themselves except in the case of the formative cells and concluded that regeneration in planarians was correlated with the number of formative cells. They regarded the formative cells as the only important source of new tissues, there being difficulty in being sure that the formative cells arose only from pre-existing formative cells and not by differentiation. Histological sections revealed an abundance of formative cells in embryonic or juvenile stages in the development of the planarian. In some sections the formative cells were observed in the process of mitosis.

Curtis and Schulze have made a diagram illustrating the history of the formative cells in the individual according to the various theories and as arising by dedifferentiation. This is reproduced in figure 6, page 58.

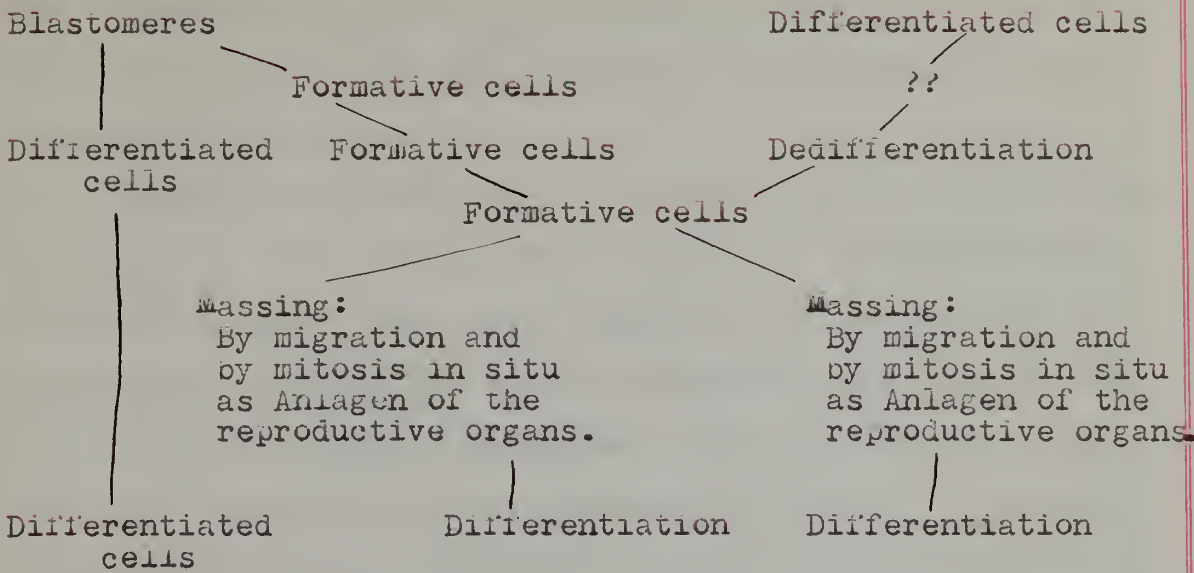

Figure 6.

Diagram of the history of the formative cells in the individual, according to the conflicting theories of formative cells as a persistent embryonic stock and as arising by de-differentiation. (After Curtis and Schulze, 1934).

ANTERIOR-POSTERIOR REGENERATION. Trembley and Spallanzani were the first to realize that, at the end of a piece of an animal from which the head has been cut, a new head develops, and that from a posterior cut surface of a piece a new posterior part regenerates. This type of regeneration is anterior-posterior regeneration.

If the anterior end is cut off at any level a new head is produced. The new worm is at first too short, the head being too near the new pharynx. However, changes take place in the region behind the head that lead to the development of new material in this part. The new head is carried farther

and farther forward until the typical relations of the parts have been formed, and until growth in the region behind the head comes to an end.

Wilson(1941) found that the regeneration of a planarian head kept in four-fifths Ringer's solution occurred in the following manner: The wound healed by the contraction of the adjacent tissues and a slow overgrowth from the cut margins of the ectodermal epithelium. The cells of the parenchyma were stimulated to divide and migrated to the surface of the wound where they accumulated as a mass of indifferent tissue. The nerve fibers from the old nerve cord probably grew into this tissue mass and exerted a formative influence.

Planarians cut transversely between the pharynx and head developed two half heads on the anterior surface when kept in dilute Ringer's solution according to Wilson. He found that they later united to form one complete head. He described the process as one whereby the wound closed by contraction. There was a healing of the cut edges of the ectodermal epithelium, which took place more slowly than by the overgrowth of the epithelium from these edges. Two pockets were thus formed in which the migrating formative cells accumulated. In the mass of new tissue thus formed, a cerebral ganglion and an eye were differentiated. The two half-heads were united by the bridge of tissue growing ventral to the wound.

Wilson (1927) described some rather unusual cases of head regeneration in Planaria. From a median longitudinal cut extending from the tail to the front of the pharynx regenerating

heads produced multiple eyed heads at the anterior angle. Other heads were produced on the lateral margin of the cut, behind the anterior angle with the axis perpendicular to the axis of the original animal. In others, the heads formed from the bridge of tissue grown across the anterior angle as a pouch extruding ventrally with the dorsal surface of the head lining the cavity.

Heads regenerated on the posterior margin of a rectangular hole cut through the body in the region of the pharynx. Tails were produced on the anterior margin of the hole, forming two individuals united by lateral strips. Heads were produced on the lateral margin of the hole with the axis perpendicular to the original axis of the animal.

Head like structures were regenerated in the new tissue formed by the closing of a cut. These structures projected dorsally as conical outgrowths, and were identified by eye pigment spots.

Compound heads with more than one anterior tip and several eye spots regenerated around the median anterior angle of the tail piece obtained by two oblique cuts extending posteriorly and laterally from a median point behind the head.

There are five classes of anterior ends produced in regenerated pieces. These are described by Buchanan (1922) as, 1) normal or those cases in which the head is the usual form of those in nature, 2) teratopthalmic, in which the shape of the head is normal, but the eyes show some degree of abnormality ranging from slight reductions in size and nearness to the

median line, to a single eye; 3) teratomorphic forms are those in which the shape of the head is abnormally reduced in size. The cephalic lobes are approximated to the median line and the eye is single and median; 4) anopthalmic is more or less anterior regeneration but has no eye, and 5) the acephalic or headless type, which has no appreciable anterior regeneration. It is merely a healed wound.

LATERAL REGENERATION. Lateral regeneration in planarians takes place readily. Morgan (1901) showed that if a worm was split in two along the middle of the body each half regenerated the missing half. This was observed to be brought about by the development of new tissue along the cut side and the extension into new parts of the outgrowth from the digestive tract. He also found that if a worm was split lengthwise into two unequal parts lateral regeneration took place. The larger piece produced material along the cut side, and into the new parts extended branches of the old digestive tract. Further observations showed that if a piece were cut from the side of a planarian, a head developed at the anterior end of the new material. At first the head was developed more to one side than anteriorly, but later changed it's position.

OBLIQUE REGENERATION. To produce oblique regeneration Morgan (1901) cut planarians obliquely. A new head appeared first to one side and not in the middle of the oblique surface on the anterior cut surface of the posterior piece. The new head formed at right angles to the cut surface. The anterior piece produced a new tail at the side of the posterior cut

surface between the old and the new parts. If a cross piece was cut and kept until the new tissues appeared over the anterior and posterior cut surfaces, then split lengthwise, a new head was developed from each piece. This new material was at first totipotent, that is, made up of formative cells. Further observations by Morgan showed that if the anterior end was cut, only a head was formed at the anterior cut surface of a posterior piece, and an intermediate region was absent. As soon as enough material from the anterior end was formed, the head appeared and began to develop. Since the tendency to produce a head approaching the maximum size was stronger than the tendency to produce as much as possible of the missing anterior end, he concluded that all the new material goes to form the head.

Morgan (1904) found that, in planarians, the shortness of a piece is a factor which enters into the character of the new part. In Planaria maculata, it has been generally agreed upon that there is a tendency for the new structure to become a head rather than a tail. He found that when the influence of polarity is removed a head appears on short cross pieces. In Planaria simplicissima, the tendency for the regenerative processes to produce a tail was stronger than to produce a head, so that two tails appeared when the polarity was reduced or removed.

<u>GRAFTS</u>. In grafts on Phagocata gracilis, Morgan (1906) cut short pieces from different parts of the worm and grafted these by the anterior surface to the anterior surface of long

pieces from different parts of another worm. If conditions of the graft were such that a part of an anterior surface was exposed, a head was regenerated from the exposed part of that surface. A single head formed, gradually grew to the size of a head of the larger component, the smaller duplicate parts of the compound worm were absorbed, and one complete worm lived.

Morgan also found that long pieces from the head region of a worm, reversed and grafted on the head region of another worm, regenerated tails at the exposed posterior surface of the shorter component of the graft.

When both long and short components of a graft were cut through the head regions, it was found that a head regenerated at the forward angle on one edge of the line of the graft, although there was no exposed anterior surface.

In dorso-ventral grafts double worms were produced. The two heads were at the line of the graft each being derived from a component of the graft. The heads regenerated at the normal place for head regeneration, but not from the free cut surface. The exposed posterior surface of the smaller component in the case of the dorso-ventral graft regenerated a tail and never closed in. It became a more or less full sized worm attached to the other worm.

INFLUENCE OF THE NERVOUS SYSTEM. When the nervous system of grafts was studied by Morgan, she found that the longitudinal nerves of the two components of the graft did not squarely unite and probably the anterior ends of longitudinal

nerves remained free.

In components yielding single headed worms where the nervous system was studied, Morgan found the longitudinal nerve trunks of the two components of the graft were connected, and that the anterior nervous system and innervation of the eyes was derived from the two components according to which part of the new head each gave rise.

In the planarian, Dendrocoelum lacteum, Goldfarb (1908) found it was improbable that the removal of the nervous system in the anterior third was responsible for the lack of regeneration of the head in the posterior pieces, because removal of that part of the nervous system did not prevent regeneration of a tail. He also found that the presence of half of this portion of the nervous system allowed the regeneration of a head. Even when the whole of the ganglion and cords were cut away regeneration took place.

The triclad turbellaria have been supposed to differ from the polyclad turbellaria in their powers of regeneration. Planaria, as has been shown, are able to regenerate complete individuals from pieces taken from any portion of the body. The polyclads, on the other hand, are unable to restore the anterior parts when the cephalic ganglia are absent. In these animals regeneration is controlled considerably by the central nervous system. Child (1904) stated there was a relation between the nervous system and regeneration in Leptoplana that might be manifested directly or indirectly. The relation might be a direct one in that the nervous stimuli constituted

the formative factors; indirectly, in that the functional conditions resulting from the rise of a part in a particular manner, as determined by it's relations with the nervous system, are the formative factors. These may be stimuli to growth or directly mechanical.

Child also found in Leptoplana that anterior regeneration, which occurred in regions anterior to the middle of the ganglia, was complete. From levels posterior to the middle of the ganglia, regeneration was incomplete. Olmstead (1922) found worms cut by transverse section posterior to the cephalic ganglion restored all missing parts. He stated that if the cephalic ganglia were injured the missing anterior parts were unable to regenerate. If the brain were injured, the cut edges healed together at about the level of the brain, and from that point remained open indefinitely.

Lateral regeneration in the presence of the ganglia was found by Child to be complete. When the ganglia were absent regeneration was still found to be complete except in the head region. The amount and rapidity of regeneration were directly proportional to the size of the part removed, especially if the removed piece was large. He found the proportion became inversed as the size of the piece was reduced.

While it is seen that the localization for the regeneration of anterior parts is undoubtedly in the cephalic ganglia, the mechanism for the restoration of the tail belongs to the body as a whole and is independent of that for restoring the head.

X-RADIATION. Bardeen and Baetjer (1904) found that by exposing planarians to the action of Roentgen rays from ten to twenty minutes a day, the powers of regeneration would be destroyed. When the anterior region of the body was removed and the rest of the worm subjected to the x-ray, no new heads were formed. Microscopical preparations showed degenerative changes had taken place, which began in the region of the head and extended slowly back. They concluded that the x-rays have a powerful inhibitive effect upon cell reproduction, which may be entirely stopped by sufficient exposure.

ANESTHETICS. Buchanan (1922) showed that the head frequency, in pieces of planarians, could be controlled by subjecting the pieces for short periods, after sectioning, to appropriate concentrations of chloretone, chloroform, chloral hydrate, ether and ethyl alcohol. An increase or decrease in the head frequency was produced with a single anesthetic by using different concentrations and varying the periods of exposure. He reported that the increase of the oxygen consumption following section, does not occur in such concentrations of anesthetics, except ethyl alcohol, and explained that the increase in the oxygen utilization in this case was due to oxidation of the alcohol and not from the stimulation. He concluded that anesthetics alter the head frequency by direct inhibition of the processes of development of the cells near the anterior cut surface.

CARBON DIOXIDE PRODUCTION. Robbins and Child (1920) reported that immediately after sectioning there was a slight

increase, which was temporary, in the carbon dioxide production of head pieces, and a very marked increase in posterior pieces. Then, in the development of a new individual from a piece, there was a considerable increase in the carbon dioxide production not only in the new outgrowths at the two ends of the animal, but in the old parts as well. After the piece had fully developed, an increase in the carbon dioxide production was found before and after feeding.

STRYCHNINE. Miller and Miller (1937) found that pieces of planarians placed in strychnine solutions showed no evidence of stimulation and did not regenerate.

SUMMARY.

The phenomenon of regeneration occurs extensively throughout the animal kingdom. From the first experiments of Trembley to the present, investigators have tried to find the cause of the phenomenon and have described the regenerative powers of many different kinds of animals. This paper deals with the process in the lower groups of invertebrates.

Sponges possess many cells which can, after dissociation and differentiation, reconstitute the whole organism from which they were separated.

In the hydra isolated cells do not unite to form a complete individual. The three body layers must be intact in fragments in order that fusion and regeneration may occur.

The hydroids, Tubularia, Pennaria, and Eudendrium exhibit wide variations in their powers of regeneration. Oxygen is an important factor for the production of hydranths in all these forms. The removal of the perisarc is necessary in Tubularia to obliterate any existing polarities in the stem, whereas in Eudendrium, the perisarc must be present for regeneration to occur. Pennaria and Eudendrium can undergo dissociation similar to the sponges. The coelenterates all possess a physiological gradient during regeneration.

The methods of inducing regeneration, and an account of the process was described in Mnemiopsis leidyi, a ctenophore. It was also found that Mnemiopsis is lacking a physiological gradient such as is present in the coelenterates.

The platyhelminthes as a group have highly specialized

powers of regeneration as was shown by various species of planarians. Regeneration in the planarians seems to be correlated with the number of formative cells in the cut piece. Even the smallest and most irregular pieces are capable of regeneration. Many abnormalities have been produced by applying varied types of stimulus to the regenerating sections, and by grafts.

Other factors, environmental, chemical, and physical, enter into the process of regeneration and have different effects in the regeneration of different animals. Light seems to be necessary for the regeneration of most of the animals. However, Eudendrium colonies will regenerate just as well in the dark. Salts and drugs seem to depress or are toxic to some of the coelenterates, as are anesthetics to the flatworms. The effects of agents upon the regenerating Tubularia have not been studied extensively. Treatment by x-radiation in both the coelenterates and flatworms results in an inhibition of the ability to differentiate new structures.

Thus, it is seen that regeneration occurs throughout the lower metazoa according to the response of the tissues to the stimulus provided, and by regulatory changes by which the piece gradually assumes normal proportions.

ABSTRACT.

The problem of regeneration has to do with the phenomenon of growth resulting in a restoration of an old form of a mutilated organism. The process, the stimuli and factors causing it, and the effect of agents upon regeneration in the lower metazoa were discussed.

The sponges, the simplest multicellular animals, can after dissociation reconstitute the whole organism again. The archaeocytes were found to have the most important role in regeneration, differentiating into gonocytes, scleroblasts, collenocytes and desmacytes, thus giving rise to the mesenchyme and skeleton of the new sponge. The pinacocytes formed the dermal membrane and lining of the canals, while the choanocytes gave rise to the flagellated chambers. Aggregates were formed by the amoeboid activity of the archaeocytes within eighteen to twenty-four hours after dissociation. On the third day after dissociation the flagellated chambers appeared, and on the fourth and fifth days, canals appeared. Development after dissociation was then completed.

Dissociated cells from different species (Microciona and Cliona) coalesce and form aggregates only with cells of their own species.

The dissociated cells will not coalesce and form aggregates in pure sodium or potassium chloride solutions. The cells were also found to be sensitive to increases in osmotic pressure.

Hydra, turned inside out, will regain their normal organization by the rearrangement of endodermal and ectodermal cells migrating in opposite directions through the mesoglea. Ectodermal or endodermal layers of hydra cultured alone do not regenerate due to the inability of one cell type to differentiate into the cell type of the other layer.

Dissociated cells of hydra will fuse and form aggregates only when the three body layers are present.

The pieces of a hydra must measure more than one-sixth of a millimeter in diameter to regenerate. Size also determines the number of tentacles, and the size of the hypostome formed on the regenerant.

Various sections of hydra and grafts also form regenerated individuals, although in some cases the results are abnormal.

The determination and organization of new polarities depended upon the rate of metabolism incorporated in the regenerating mass.

In Tubularia, removal of the perisarc stimulates regeneration, as the cut end is then bathed with more oxygen. The more coenosarc exposed to oxygen the greater is the activation to form hydranth tissue. When there is an increase in the oxygen consumption there is also an increase in the rate of regeneration. The hydranth forms in relation to the oxygen supply with the oral end of the regenerant at the point of highest oxygen tension.

The rate of regeneration can be measured at any level under various conditions when the formula $R=\pi r^2 L/t$ is used.

Exposure of the coenosarc to sea water obliterates the already existing gradients in the stem and thus the regenerating coenosarc fragments form new polarities.

The theory of dominance seems to be best explained by the transportation of substances theory, in which there are differences in the stem,"E", present in highest concentration at the distal end and lowest at the proximal end. There is also another factor in the stem "S", which may or may not inhibit regeneration depending on the rate of regeneration when "E" is forming new hydranths.

Eudendrium and Pennaria cells fuse after dissociation in much the same manner as sponges and hydra. Light is essential for the regeneration of hydranths in Pennaria, but not for Eudendrium.

Direct x-radiation inhibited regeneration of Pennaria, but screened colonies connected to those colonies radiated, did regenerate new hydranths.

Podocoryne and Hydractinia are also capable of regenerating polyps. Gonionemus shows incomplete regeneration.

Mnemiopsis leidyi, a ctenophore, regenerated plate rows, canal connections, and apical organs, depending on the manner in which they were cut. There was found to be no physiological gradient in Mnemiopsis.

Polychoerus is the simplest flatworm to stimulate to regenerative activity as these worms have no central nervous system to influence regeneration.

Many types of regeneration have been produced from

stimulation of planarians to regenerate. The smallest and most irregular pieces have the capability of forming new individuals. The extensive powers of regeneration of this group are due to the localization and differentiation of the formative cells, which are found in the mesoderm.

The polyclads will regenerate complete individuals from pieces taken from any part of the body. The triclads will restore missing anterior parts only when the cephalic ganglia are present.

Anesthetics and strychnine inhibit regeneration in the flatworms as does exposure to x-radiation.

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